

complete mixing of the liquids. Proceed as directed in paragraph (e) of this section.

(e) *Procedure.* Cover the top of each centrifuge tube with a plastic cap. Punch a small hole in the top of each cap to allow vapor to escape. Place the tubes in a 100° C. drying oven for 2.5 hours. Remove the tubes from the oven and allow to cool. Take the plastic cap from each tube and replace with the glass stopper. Centrifuge 10-15 minutes at 2,000-2,500 r.p.m. to separate the white solid from the liquid in the tube. Inject 0.5 microliter of the clear liquid into the gas chromatograph. Use the conditions and materials listed in paragraphs (a), (b), and (c) of this section. The conditions should be adequate to maintain a stable baseline and provide at least 60 percent deflection of the recorder scale by the clindamycin peak. The resolution of the peaks should be complete. The elution order is: Internal standard, clindamycin, and epiclindamycin (if present). Calculate the clindamycin content as directed in paragraph (f) of this section.

(f) *Calculations.* Calculate the clindamycin content of the sample as follows:

$$\text{Micrograms of clindamycin per milligram} = \frac{R_u \times W_s \times f}{R_s \times W_u}$$

where:

$R_u$ =Area of the clindamycin sample peak (at a retention time equal to that observed for the clindamycin standard)/Area of internal standard peak;

$R_s$ =Area of the clindamycin standard peak/Area of internal standard peak;

$W_s$ =Weight of the clindamycin working standard in milligrams;

$W_u$ =Weight of the sample in milligrams;

$f$ =Potency of the clindamycin working standard in micrograms per milligram.

**§ 436.303 Clindamycin content of clindamycin palmitate hydrochloride by vapor phase chromatography.**

(a) *Equipment.* Gas chromatograph equipped with a flame ionization detector: Hewlett-Packard 7606<sup>4</sup> or equivalent.

(b) *Reagents.* (1) Acetic anhydride, reagent grade.

(2) Pyridine, reagent grade.

(3) Chloroform, reagent grade.

(4) Internal standard: Prepare a solution containing 5 milligrams of cholesteryl benzoate per milliliter in chloroform.

(c) *Typical conditions.* (1) Column: 6 feet × 2 millimeters ID, glass, with 1 percent UC-W98 on Chromosorb WHP (80/100 mesh) or equivalent.

(2) Temperatures: Column 275° C.; detector 290° C.; injection port 280° C.

(3) Carrier gas: Helium approximately 60 milliliters per minute.

(4) Detector: Hydrogen flame ionization—hydrogen at 12 pounds per square inch, air at 32 pounds per square inch.

(5) Sensitivity: 1,000; attenuation, 16;  $1 \times 10^{-9}$  amperes.

(d) *Preparation of clindamycin palmitate hydrochloride sample and working standard solutions.* Accurately weigh approximately 15 milligrams of both the sample and the working standard into separate glass-stoppered, conical 15-milliliter centrifuge tubes. Add 1.0 milliliter of internal standard solution, 1.0 milliliter of pyridine, and 0.5 milliliter of acetic anhydride to each tube. Agitate the tubes to insure dissolution and complete mixing of the liquids. Proceed as directed in paragraph (e) of this section.

(e) *Procedure.* Cover the top of each centrifuge tube with a plastic cap. Punch a small hole in the top of each cap to allow vapor to escape. Place the tubes in a 100° C. drying oven for 2.5 hours. Remove the tubes from the oven and allow to cool. Take the plastic cap from each tube and replace with the glass stopper. Centrifuge 10-15 minutes at 2,000-2,500 r.p.m. to separate the white solid from the liquid in the tube. Inject 1 microliter of the clear liquid into the gas chromatograph. Use the conditions and materials listed in paragraphs (a), (b), and (c) of this section. The conditions should be adequate to maintain a stable baseline and provide at least 40 percent deflection of the recorder scale by the clindamycin palmitate peak. The resolution of the peaks should be complete. The internal standard will be eluted before the clindamycin palmitate. Calculate the clindamycin content as directed in paragraph (f) of this section.

<sup>4</sup> Available from: Hewlett Packard Co., P.O. Box 301, Loveland, CO 80537.

(f) *Calculations.* Calculate the clindamycin content of the sample as follows:

$$\text{Micrograms of clindamycin per milligram} = \frac{R_u \times W_s \times f}{R_s \times W_u}$$

where:

$R_u$ =Area of the sample peak (at a retention time equal to that observed for the clindamycin palmitate hydrochloride standard)/Area of internal standard peak;

$R_s$ =Area of the clindamycin palmitate hydrochloride standard peak/Area of internal standard peak;

$W_s$ =Weight of the clindamycin palmitate hydrochloride working standard in milligrams;

$W_u$ =Weight of the sample in milligrams;

$f$ =Micrograms of clindamycin activity per milligram of clindamycin palmitate hydrochloride working standard.

#### § 436.304 Clindamycin phosphate vapor phase chromatography.

(a) *Equipment.* Gas chromatograph equipped with an electronic integrator and with a flame ionization detector that has a sensitivity of at least  $1 \times 10^{-10}$  amperes: Hewlett-Packard 7600<sup>4</sup> or equivalent.

(b) *Reagents.* (1) Trifluoroacetic anhydride.

(2) Intestinal alkaline phosphatase.

(3) pH 9.0 borate buffer: Transfer 3.1 grams of boric acid into a 1-liter volumetric flask containing 500 milliliters of water, mix, and add 21 milliliters of 1.0*N* sodium hydroxide and 10 milliliters of 0.1*M* magnesium chloride. Dilute to volume with water and mix well.

(4) Internal standard: Prepare a chloroform solution containing approximately 0.45 milligram hexacosane per milliliter.

(5) Anhydrous sodium carbonate.

(c) *Typical conditions.* (1) Column: 2 feet  $\times$  3 millimeters ID, glass, with 1 percent SE-30 on Diatoport S (80/100 mesh), or equivalent.

(2) Temperatures: Column, 180° C., detector, 215° C., injection port, ambient temperature.

(3) Carrier gas: Helium approximately 60 milliliters per minute.

(4) Detector: Hydrogen flame—hydrogen flow at 40 milliliters per minute. Air flow at 400 milliliters per minute.

(5) Sensitivity:  $1 \times 10^{-9}$  amperes.

(d) *Preparation of clindamycin phosphate sample solution.* Accurately weigh approximately 12 milligrams of the clindamycin phosphate sample into a 50-milliliter glass-stoppered centrifuge tube. Pipet 25 milliliters of the pH 9.0 borate buffer into the centrifuge tube. Add 10 milliliters chloroform and shake vigorously for 15 minutes. Centrifuge the resulting mixture and pipet a 20-milliliter aliquot of the aqueous phase into a 35-milliliter centrifuge tube. Add a weighed amount of intestinal alkaline phosphatase equivalent to 50 units of activity<sup>5</sup> and allow the solution to stand until the enzyme has completely dissolved. Place the tube into a water bath at 37° C.  $\pm$  2° C. for 2.5 hours. After the 2.5-hour hydrolysis, allow the solution to cool and proceed as directed in paragraph (f) of this section.

(e) *Preparation of the clindamycin hydrochloride standard solution.* Accurately weigh approximately 9 milligrams of the clindamycin hydrochloride working standard into a 35-milliliter glass-stoppered centrifuge tube and dissolve in 20 milliliters of pH 9.0 borate buffer. Proceed as directed in paragraph (f) of this section.

(f) *Procedure.* Add 10 milliliters of the internal standard solution to each sample and standard solution. Shake the centrifuge tubes vigorously for 30 minutes and centrifuge. Remove the aqueous layer and discard. Shake the tubes again; mix in an ultrasonic mixer for 2 minutes, then centrifuge. No emulsion should be present at this stage. Remove the remaining aqueous layer by suction and transfer a 3-milliliter aliquot of the chloroform layer to a 1-dram tablet vial containing approximately 1 gram of anhydrous sodium sulfate. Swirl the vial to dry the chloroform and transfer a 1-milliliter aliquot to another 1-dram tablet vial. Using a 0.25-milliliter pipet, add 0.25 milliliter of trifluoroacetic anhydride to

<sup>5</sup>Defined such that 50 units hydrolyzes at least 20 micromoles of a clindamycin phosphate authentic sample under the assay conditions described in this section.

<sup>4</sup>See footnote 4 to § 436.303(a).